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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 31656.15-2022

National Food Safety Standards Determination of Mebendazole and Its Metabolite Residues in Aquatic Products-Liquid Chromatography-Tandem Mass Spectrometry

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1.Structure and Drafting Rules for Standardization Documents"

drafting.

This document is published for the first time.

National Food Safety Standards

Determination of residues of mebendazole and its metabolites in aquatic products

Liquid chromatography-tandem mass spectrometry

1 Scope

This document specifies the sample preparation and procedures for the detection of mebendazole and its main metabolites, hydroxy-mebendazole and amino-mebendazole residues in aquatic products.

Liquid chromatography-tandem mass spectrometry method.

This document is applicable to the residues of mebendazole and its main metabolites hydroxy-mebendazole and amino-mebendazole in edible tissues of fish, shrimp and turtle

detection.

2 Normative references

The content in the following documents constitutes the essential provisions of this document through normative references in the text. Among them, the dated reference documents,

Only the version corresponding to the date applies to this document; for undated references, the latest version (including all amendments) applies to this document

document.

GB/T 6682 Water rules and test methods for analytical laboratories

GB/T 30891-2014 Sampling Specifications for Aquatic Products

3 Terms and Definitions

This document does not have terms and definitions that need to be defined.

4 principles

The residual mebendazole, hydroxymesbendazole and aminomesbendazole in the sample were extracted with ethyl acetate, degreased with n-hexane, and analyzed by liquid chromatography-

Detection by tandem mass spectrometry and quantification by internal standard method.

5 Reagents and materials

Unless otherwise specified, all reagents are of analytical grade, and the water is first-class water in accordance with GB/T 6682.

5.2 Preparation of solution

5.2.1 50mmol/L disodium hydrogen phosphate solution. Take 8.95g of disodium hydrogen phosphate, dissolve it in water and dilute to 500mL.

5.2.2 0.1% formic acid solution. take 0.5mL formic acid, add water to dissolve and dilute to 500mL, mix well.

5.2.3 Methanol-0.1% formic acid solution (50.50, V/V). Take methanol and 0.1% formic acid solution and mix them in equal volumes.

5.3 Standards

Mebendazole (MBZ), content >=99.0%; hydroxy mebendazole (MBZ-OH), content >=99.0%; amino mebendazole (MBZ-OH)

NH2), content >=99.0%; deuterated mebendazole (MBZ-D3), content >=99.5%; deuterated hydroxy mebendazole (MBZ-OH-D3), content

>=99.0%. See Appendix A for details.

5.4 Preparation of standard solution

5.4.1 Standard stock solution (100 μg/mL). Take 10 mg of each standard substance of mebendazole, hydroxy-mesbendazole and amino-mesbendazole, and accurately weigh

Dissolve with 10mL dimethyl sulfoxide, add methanol to dilute to 100mL volumetric flask, shake well. Store below -18°C, valid for 3

month.

5.4.2 Mixed standard working solution. Accurately measure 1 mL each of standard stock solutions of mebendazole, hydroxy-mesbendazole and amino-methbendazole,

Alcohol-0.1% formic acid solution was diluted to prepare a mixed standard working solution with concentrations of 1000ng/mL, 100ng/mL and 10ng/mL.

Use it now.

5.4.3 Internal standard stock solution (100 μg/mL). Take 10 mg of deuterated mebendazole and deuterated hydroxymesbendazole standard substances, weigh them accurately,

Dissolve in 10mL dimethyl sulfoxide, dilute with methanol to 100mL volumetric flask, shake well. Store below -18°C, valid for 3

month.

5.4.4 Mixed internal standard working solution. Accurately measure 1 mL of internal standard stock solution of deuterated mebendazole and deuterated hydroxymesbendazole, and use methanol-

The 0.1% formic acid solution was diluted into a mixture containing deuterated mebendazole and deuterated hydroxymesbendazole at concentrations of 50 ng/mL and 10 ng/mL, respectively.

Internal standard working solution. Prepare now.

5.5 Materials

Microporous nylon filter membrane. 0.22 μm.

6 Instruments and equipment

6.1 Liquid chromatography-tandem mass spectrometry (LC-MS/MS). with electrospray ionization source (ESI).

7.1 Preparation of samples

Prepare samples according to the requirements of Appendix B of GB/T 30891-2014.

a) Take the homogenized test sample as the test material;

b) Take the homogenized blank sample as the blank sample;

c) Take the homogenized blank sample, add the standard working solution of appropriate concentration, and add the sample as the blank.

7.2 Storage of samples

Store below -18°C.

8.1 Extraction

Take 2 g of the test material (accurate to +/-0.05 g), put it into a 50 mL centrifuge tube with stopper, accurately add 100 μL of mixed internal standard working solution, and vortex

2min, add 5mL of 50mmol/L disodium hydrogen phosphate solution, vortex for 1min, add ethyl acetate 6mL, vortex for 1min,